LWT - Food Science and Technology 87 (2018) 47–53

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of microwaves and ultrasound on bioactive compounds and microbiological quality of blackberry juice

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A R T I C L E I N F O

Article history:
Received 24 March 2017
Received in revised form
20 July 2017
Accepted 19 August 2017
Available online 23 August 2017

Keywords:
Blackberry juice
Microwaves
Ultrasound
Polyphenols
Microbiological quality

A B S T R A C T

Blackberry mash added with citric acid (0–500 mg/kg) was treated with microwaves (0–60 s), and the resulting juice was evaluated to determine the effects on bioactive compounds and antioxidant activity using surface response methodology. This methodology allowed the selection of the parameters for the microwave treatment and concentrations of citric acid that yielded the highest content of total polyphenols, monomeric anthocyanins and antioxidant activity of the juice. Mash samples were sonicated (40% amplitude, 20 KHz, 10 min) and stored at 5 °C + 1 °C for a month. Then the contents of total polyphenols and monomeric anthocyanins, as well as microbiological quality were analyzed. Analysis of polyphenols was carried out by UPLC-DAD in the raw juice and the sample selected by surface response methodology and treated with ultrasound. The highest levels of polyphenols and monomeric anthocyanins in juice were obtained at a time of 60 s of microwave processing and a concentration of 500 mg/kg citric acid in the blackberry mash. Phenolic acids, flavonols and cyanidin were detected in both samples. During storage, juice processed with ultrasound retained more than 90% of monomeric anthocyanins and showed a significant microbial load reduction, agreeing with the quality parameters established by the Mexican standards. This study highlights the potential application of microwaves and ultrasound to increase the functional value of blackberry juice.

1. Introduction

Nowadays consumers have a particular interest in beverages that provide bioactive compounds with healthful effects, such as anthocyanins, which are polyphenolic compounds with anti-inflammatory, anti-carcinogenic, and antioxidant properties (Dai & Mumper, 2010; Zafra-Stone et al., 2007). Berries, used in the production of beverages, are a good source of these compounds. However, it has been reported that industrial processing decreases the content of anthocyanins in the final product (Hager, Howard, & Prior, 2008; Howard, Prior, Liyanage, & Lay, 2012). Since anthocyanins have so many beneficial effects on human health, it is relevant to explore the application of alternative technologies in juices prepared from anthocyanin-rich fruits – like blackberry – to increase their content of natural antioxidants and functional value. An important step in the industrial preparation of fruit juices is blanching. Previous reports suggest that microwave blanching may be used to obtain fruit juice with a high quantity of polyphenolic compounds (Gerard & Roberts, 2004), since microwaves produce a quick and uniform heating, minimizing changes in flavonoids such as anthocyanins (De Ancos, Cano, Hernandez, & Monreal, 1999).

On the other hand, it is well known that the traditional process for juice production induces changes in anthocyanins and increases polymerized compounds in berry juices (Brownmiller, Howard, & Prior, 2008; Li, Walker, & Faubion, 2011). It has been reported

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http://dx.doi.org/10.1016/j.lwt.2017.08.059

that low pH values and temperature decrease anthocyanin polymerization reactions during storage. Acidic conditions keep anthocyanins in the form of red flavylium cation, which plays an important role in the antioxidant capacity and bioavailability of anthocyanins (Lapidot, Harel, Akiri, Granit, & Kanner, 1999; Pacheco-Palencia, Mertens-Talcott, & Talcott, 2010; Van Acker et al., 1996). In addition, organic acids such as citric acid favor the presence of monomeric anthocyanins and decrease polymerization (Li et al., 2011; Nicoué, Savard, & Belkacemi, 2007).

Another important step involved in the food processing is pasteurization, a thermal treatment widely used in juice industry to obtain a safe product from the microbiological point of view. However, anthocyanins and many bioactive compounds are degraded by traditional pasteurization processes. Several authors report that ultrasound is an alternative technology to traditional thermal treatments in the reduction of microbial load (Aadil, Zeng, Han, & Sun, 2013) and to minimize changes in bioactive compounds such as anthocyanins (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009). Give the above, a combination of microwaves and ultrasound represents a promising new technology to improve the extraction of polyphenols during the blanching of fruit mash with microwaves, while ultrasound processing could be applied to the resulting juice for microbial inactivation, as well as to maintain the functional value over the shelf life of the product. Therefore, the aim of the present study was to evaluate the combined effect of microwaves and ultrasound to enhance the functional value of blackberry juice. The first part of this research was to evaluate the combined effect of microwave processing and the addition of citric acid of blackberry mash on the content of polyphenols, anthocyanins, color density, percent polymeric color and antioxidant capacity of blackberry juice, using an experimental design based on surface response methodology. The second part focused in studying the effect of ultrasound on the chemical composition and microbiological quality of blackberry juice during storage in refrigeration, as compared to the initial conditions analyzed before microwave blanching and citric acid addition.

2. Materials and methods

2.1. Material

Blackberries (*Rubus americanus* (Pers.) Britton) at ripe stage were obtained from a local market in Xalapa, State of Veracruz, Mexico. Frozen fruits were placed in plastic trays and stored at −20 °C.

2.2. Microwaves processing

In the present study, microwaves were used to blanch blackberry mash. Blackberries were thawed at 20 °C and crushed using a mortar. The resulting blackberry mash (50 g) was placed on petri dishes (10 cm diameter x 1 cm height) and heated in a microwave oven (Panasonic, 2450 MHz, 453 W). Microwave energy (E) was calculated for each sample according to the Buffler method (Buffler, 1993, pp. 157–159) using equation $E = Wt/m$, where W is microwave oven power; t is time of microwave exposure, and m = quantity of sample (Ortiz, Dorantes, Galindez, Guzmán, 2003). Immediately after the thermal treatment, the surface temperature of each sample was recorded with a Rayinger ST non-contact thermometer. After heating, the samples were cooled in ice water until the temperature dropped to 20 °C ± 1 °C. The juice was obtained by centrifugation.

2.3. Response surface methodology

Response surface methodology was applied to establish the effect of microwave time (s) and citric acid (mg/kg) on the polyphenols content (gallic acid equivalent, mg/L), monomeric anthocyanins (cyanidin-3-glucoside equivalent, mg/L), color density, percent polymeric color and antioxidant capacity by DPPH and FRAP assay (mM trolox equivalent) of blackberry mash. The levels of independent variables microwave (0–60 s) and citric acid (0–500 mg/kg) were selected according to preliminary experiments. A central composite design of 13 runs with five replicate runs at center point was performed. The statistical data analysis and response surface plots were carried out using a Design Expert statistical package 10.0. Experimental values were fitted to a second-order polynomial model and regression coefficients were obtained.

2.4. Ultrasound processing

The blackberry juice sample with the highest content of total polyphenols and anthocyanins, as showed by the surface response analysis, was selected for the ultrasound treatment. 80 mL of this juice were processed in an ultrasonic homogenizer at frequency of 20 KHz and power of 750 W (Cole-Palmer Instrumental Company, VCX-750, USA) with a 13 mm diameter probe, operating at amplitude of 32 μm with 5 s on and 5 off pulse during a time of 10 min. The probe was submerged 25 mm deep into the juice. The juice was placed into a water bath at 20 °C to control temperature. Then, the juice was stored at 5 °C ± 1 °C during 30 days in an amber glass container. The ultrasound conditions for the present study were selected according to previous reports dealing with the application of ultrasound in berry juices (Tiwari, O'Donnell, et al., 2009; Tiwari, Cullen, & O'Donnell, 2009).

2.5. Total polyphenols

Total polyphenols content was estimated by the Folin-Cicalteu assay as described by Singleton and Rossi (1965). The juice was mixed with 1N Folin-Ciocalteau reagent and then saturated sodium carbonate solution and water were added to the mixture. After 60 min, the absorbance was measured at 760 nm. A calibration curve was performed with gallic acid. The concentration of total phenolics was expressed as mg of gallic acid equivalent per liter of juice (mg GAE/L). All samples were prepared by triplicate.

2.6. Monomeric anthocyanins, color density and percent polymeric color analysis

The content of monomeric anthocyanins was evaluated using the pH differential method (Giusti & Wrolstad, 2001). The absorbance was measured in a lambda 35 UV-VIS spectrophotometer (Perkin Elmer, Inc. Shelton, CT, USA) at 515 and 700 nm. Juice samples were dissolved with 0.025 M potassium chloride buffer, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5. Then the anthocyanins content was expressed as mg of cyanidin-3-glucoside equivalent per liter of juice (mg C3GE/L), using the extinction coefficient of 26,900 L x cm^{-1} x mol^{-1}, and a molecular weight of 449.2 g/mol. For the percent polymeric color and color density analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL of diluted sample, and 0.2 mL of distilled water was added to 2.8 mL diluted sample (control). Samples were recorded at 420 nm, 515 nm, and 700 nm.